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Studies of the toxicity of Matacil® 180 F Concentrate, its formulations, and their components to aquatic fauna

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STUDIES ON THE TOXICITY OF MATACIL^{R} 180 F CONCENTRATE, ITS FORMULATIONS, AND THEIR COMPONENTS TO AQUATIC FAUNA

by

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ABSTRACT

Fingerling rainbow trout (Salmo gairdneri) were used to determine the acute lethal toxicity of Matacil 180 F, of its proposed operational formulations, and of the components of the formulations. It was concluded that the toxicity of the 180 F concentrate and the water formulation was mainly due to aminocarb and not to the various adjuvants. For the oil formulation, 63% of the toxicity could be attributed to aminocarb and 37% to diluent oil 585.

The acute toxicity of the oil and of the water formulations was also investigated using a variety of aquatic invertebrates. The fresh water Cladoceran Daphnia pulex was the most sensitive of the three invertebrates tested, with 48-hour EC50's for immobilization of 0.38 mg/L for the oil formulation and 1.1 mg/L for the water formulation. The fresh water clam Anodonta sp. was intermediate in sensitivity with 96-hour LC50's of 260 mg/L for both the oil and the water formulations. The least sensitive species tested was the sea water mussel Mytilus edulis, which had 96-hour LC50's greater than 1,000 mg/L for the two formulations. The attachment of mussels to substrates by their byssal threads was inhibited by concentrations of 114 mg/L of the oil formulation and by 550 mg/L of the water formulation.

A 30-day experiment to determine the uptake and elimination of aminocarb by the mussel, Mytilus edulis, was conducted with the water formulation of Matacil 180 F. Bioconcentration factors ranged from 1.5 to 2.6 for various exposure times up to 30 days and the mussels eliminated, or metabolized, the aminocarb in less than 24 hours when transferred to clean-flowing sea water. Bioconcentration factors equilibrated at 1.5, regardless of exposure concentration. Bioconcentration of the particulate aminocarb in the Matacil 180 F formulations is not likely to present a hazard to aquatic filter feeding organisms.

It was concluded that the toxicity of Matacil 180 F and its formulations to the non-target organisms tested were less severe than the toxicity of Matacil 180 D and its formulations. If operational dosage rates were similar, the Matacil 180 F would have a greater margin of safety, and so would be the preferable operational insecticide.

Résumé

Des alevins de truite arc-en-ciel (Salmo gairdneri) de la grosseur du doigt ont servi à déterminer la toxicité létale aiguë du Matacil 180 F, de ses préparations opérationnelles proposées et des éléments de ces dernières. On est arrivé à la conclusion que la carbylamine, et non les divers adjuvants, était surtout responsable de la toxicité du concentré 180 F et de la préparation à l'eau. Dans la préparation à l'huile, 63% de la toxicité serait attribuable à la carbylamine et 37% à l'huile 585 de dilution.

La toxicité aiguë des préparations à l'huile et à l'eau a été aussi testée sur divers invertébrés aquatiques. Le cladocère d'eau douce, Daphnia pulex, est le plus sensible des trois invertébrés étudiés, 50% des sujets étant immobilisés après 48 h à une concentration effective de 0,38 mg/L de la préparation à l'huile et 1,1 mg/L de la préparation à l'eau. Le bivalve d'eau douce, Anodonta sp., a une sensibilité intermédiaire, avec une CL50 après 96 h de 260 mg/L de l'une ou l'autre préparation. L'espèce testée la moins sensible est la moule marine, Mytilus edulis, qui a une CL50 après 96 h dépassant 1 000 mg/L des deux préparations. La fixation des moules au substrat à l'aide des fils de leur byssus est inhibée par des concentrations de 114 mg/L de la préparation à l'huile et de 550 mg/L de la préparation à l'eau.

Une expérience de 30 jours a été menée avec la préparation aqueuse du Matacil 180 F en vue de déterminer l'accumulation et l'élimination de la carbylamine par la moule, Mytilus edulis. Pour diverses périodes d'exposition allant jusqu'à 30 jours, les facteurs de bioconcentration varient de 1,5 à 2,6. On constate en outre que les moules éliminent ou métabolisent la carbylamine en moins de 24 h lorsque transférées en eau de mer fraîche en circulation. Les facteurs de bioconcentration s'équilibrent à 1,5 sans égard à la concentration

d'exposition. La bioconcentration de la carbylamine particulaire dans les préparations de Matacil 180 F ne présente probablement pas de danger pour les organismes aquatiques se nourrissant par filtration.

Nous concluons que la toxicité du Matacil 180 F et de ses préparations pour les organismes non-cibles testés est moins sévère que celle du Matacil 180 D et de ses préparations. A des dosages opérationnels identiques, le Matacil 180 F offre une marge de sécurité plus grande et, pour cette raison, est préférable comme insecticide opérationnel.

TABLE OF CONTENTS

		Page
	ACKNOWLEDGEMENTS	i
	ABSTRACT	ii
	RESUME	iv
	TABLE OF CONTENTS	vi
	LIST OF TABLES	vii
	LIST OF FIGURES	viii
1.	INTRODUCTION	1
2.	METHODS AND MATERIALS	2
2.1	General	2
2.2	Rainbow Trout Toxicity Tests	5
2.3	Aquatic Invertebrate Toxicity Tests	6
2.4	Uptake and Elimination by Mussels	8
3.	RESULTS	11
3.1	Rainbow Trout Toxicity Tests	11
3.2	Aquatic Invertebrate Toxicity Tests	11
3.3	Uptake and Elimination by Mussels	16
4.	DISCUSSION	22
5.	CONCLUSIONS	28
	REFERENCES	29
	APPENDICES	33

LIST OF TABLES

Table		Page
1	SUMMARY OF RESULTS OF THE RAINBOW TROUT TOXICITY TESTS	12
2	MEASURED AMINOCARB LEVELS (mg/L) IN TEST SOLUTIONS FOR THE RAINBOW TROUT TOXICITY TESTS	13
3	SUMMARY OF THE ACUTE TOXICITY TESTS WITH AQUATIC INVERTEBRATES	14
4	MEASURED AMINOCARB LEVELS (mg/L) IN TEST SOLUTIONS FOR THE INVERTEBRATE TOXICITY TESTS	15
5	LEVELS OF AMINOCARB IN WATER (mg/L) AND TISSUE (µg/g) THROUGHOUT THE BIOACCUMULATION EXPERIMENT WITH MUSSELS (*Mytilus edulis*)	17
6	LEVELS OF AMINOCARB IN MUSSEL TISSUE (µg/g) AFTER VARIOUS EXPOSURES AND DEPURATION TIMES	21
7	CONTRIBUTIONS TO THE LETHALITY TO RAINBOW TROUT OF MATACIL 180 F CONCENTRATE, AND ITS FORMULATIONS, BY THEIR COMPONENT CHEMICALS	23
8	SUMMARY OF TOXICITY TEST RESULTS EXPRESSED AS MEASURED AMINOCARB IN mg/L	26

LIST OF FIGURES

Figure		Page
1	SCHEMATIC DESIGN FOR EXPERIMENT ON UPTAKE OF AMINOCARB BY MUSSELS	10
2	MEASURED VALUES OF AMINOCARB IN WATER AT VARIOUS TIMES DURING THE UPTAKE AND ELIMINATION EXPERIMENT	18
3	MEASURED VALUES OF AMINOCARB IN MUSSEL TISSUE AT VARIOUS TIMES DURING THE UPTAKE AND ELIMINATION EXPERIMENT	19
4	BIOCONCENTRATION FACTOR OF AMINOCARB IN MUSSEL TISSUE AT VARIOUS TIMES DURING THE UPTAKE AND ELIMINATION EXPERIMENT	20

1. INTRODUCTION

Aminocarb (4-(dimethylamino)-3-methylphenyl methylcarbamate) is the active ingredient in one of the formulations used since 1975 in forest spray operations to control spruce budworm in New Brunswick, Canada. Mobay Chemical Corporation has produced a new aminocarb product known as Matacil 180 F oil flowable concentrate. This new product differs from the presently used Matacil 180 D oil soluble concentrate in that the aminocarb is present as air-milled particles (2-3 µm diameter) in an oil suspension and contains no nonylphenol, whereas the 180 D consists of technical aminocarb dissolved in the solvent nonylphenol and diluted with insecticide diluent 585 (Szeto and Holmes, 1982).

Because of the lack of information concerning the toxicity of this new concentrate, the Environmental Protection Service, Atlantic Region, carried out a toxicity study on Matacil 180 F, its formulations, and their components using fish and aquatic invertebrates. The Matacil 180 F contains finely-milled particulate aminocarb and it was felt that filter feeding organisms such as bivalves might concentrate the insecticide. Since many of these organisms are eaten by man, an experiment to determine uptake and elimination of aminocarb by the edible mussel Mytilus edulis was conducted. This report summarizes the results of these investigations.

2. METHODS AND MATERIALS

2.1 General

Matacil 180 F concentrate is intended to be mixed with other ingredients to make either a water or an oil formulation for actual use in the field. The test formulations were mixed to duplicate operational formulations as follows:

(a) Water Formulation - The following ingredients were mixed thoroughly: 21 parts of 180 F concentrate; 1 part of Atlox 3409 F; and 58 parts of fresh water (by volume).

(b) Oil Formulation - The following ingredients were mixed thoroughly: 1 part of 180 F concentrate; and 2.8 parts of diluent oil 585 (by volume).

The components of the formulations were obtained either from Chemagro Ltd., Mississauga, Ontario, or from Forest Protection Limited, New Brunswick. The formulations were made up on an as-needed basis immediately prior to each bioassay.

Chemical analyses were conducted on samples from test solutions to determine actual aminocarb test concentrations. Chemical analyses for aminocarb were also conducted on tissue samples from the uptake and elimination experiment using documented analytical methods (Anonymous, 1979). A detailed description of analytical techniques is contained in Appendix 1. The detection limit for aminocarb was 1.0 µg/L in water and 0.05 µg/g in tissue samples.

The freshwater used in these tests was limed, fluoridated and chlorinated Dartmouth City water which was passed through activated charcoal filters and ultra-violet sterilizers for dechlorination. The

water is soft, slightly alkaline, low in heavy metal content and has limited buffering capacity. Additional characteristics of this water are presented in Appendix 2. In the Daphnia pulex tests the previously described water was used to culture the test organisms, and water taken from an active culture was screened to remove the animals, and was used for dilution and control water in the toxicity tests.

For the tests with the mussel Mytilus edulis, Bedford Basin sea water was used. A (1:1) mixture of unfiltered and sand and gravel filtered sea water was used to hold the animals, and also utilized in the 30 day uptake and depuration tests. Sand and gravel filtered sea water was used in the 4 day acute exposures, while the 4 day recovery from these exposures was carried out in a flow-through mixture of filtered and unfiltered sea water. The Bedford Basin sea water used in these tests was taken from a depth of 10 metres. Bedford Basin sea water has been characterized by investigators from the Bedford Institute of Oceanography, and the results have been summarized (Doe and Wells, 1978). The salinity of the water ranges from about 29 to 31 parts per thousand.

For the tests conducted on the water formulation and on the emulsifier Atlox 3409 F, the test materials were added to the test tanks and stirred. For tests conducted on Matacil 180 F, the oil formulation, diluent oil 585 and Sunspray 7-N oil (a component of the Matacil 180 F), appropriate amounts of the test materials were added to 500 mls of water in a 2-speed Waring commerical blender and blended for one minute at high speed before being added with vigorous stirring to the test tanks.

The photoperiod for holding and tests with rainbow trout (Salmo gairdneri), Anodonta sp. and Mytilus edulis was 12 h light; 12 h dark. The photoperiod for holding and testing with Daphnia pulex was 16 h light; 8 h dark.

For the acute toxicity tests with rainbow trout and aquatic invertebrates, test organisms were not fed for at least 24 hours before a test and during the test exposures. Mussels used for the 30 day uptake and elimination experiment were supplied with unfiltered Bedford Basin sea water which contained a natural plankton flora and fauna, providing adequate food for both the exposure and clean water depuration phases of the experiment.

All acute toxicity tests used the general principles of acute toxicology as outlined in Sprague (1969; 1970). The results are expressed either as the concentration for 50% mortality (LC50), or as the concentration which shows the measured sub-lethal effect in 50% of the individuals tested (EC50). These estimates were derived from response data using a modified BMD 03S program (Dixon, 1970) where two or more partial responses were available; or graphically according to the method of Litchfield and Wilcoxon (1949) where fewer than two partial responses were available. A lack of resources and time prevented measurement of aminocarb concentrations in some test solutions. The LC50 and EC50 estimates are expressed as nominal concentrations. All results for the uptake and elimination experiments are expressed as measured values of aminocarb.

Measured values of aminocarb were compared with expected values. The expected values of aminocarb were calculated by multiplying the nominal concentration of the test substance by the percentage of aminocarb in the test substance. These values were:

(a) Matacil 180 F - first batch (used in all acute toxicity tests) - 24.3% aminocarb;

(b) Oil formulation (used in all acute toxicity tests) - 7.23% aminocarb;

(c) Water formulation (used in all acute toxicity tests) - 6.04% aminocarb;

(d) Water formulation (uptake experiments only) - 4.87% aminocarb*.

2.2 Rainbow Trout Toxicity Tests

Fingerling rainbow trout (Salmo gairdneri) were obtained from Goossen's Trout Farm, Otterville, Ontario. All the fish used were from the same batch, and they were held at 15 ± 2°C prior to the tests. Average length ranged from 4.8 to 6.0 cm, and average weight ranged from 1.5 to 2.3 g (Appendix 3). Fish loading density ranged from 0.43 to 0.67 L/g/d (Appendix 3). All tests were 40 litre, static, acute lethal toxicity tests with 10 animals per test tank. Tests were 96 hours duration, and test solutions were aerated lightly at 200 ml air/minute.

Temperature, dissolved oxygen and pH were measured daily. Values ranged from 14.5 to 15.5°C for temperature; 5.5 to 10.6 mg/L for dissolved oxygen; and 6.4 to 7.4 for pH (Appendix 4). Duplicate tests were carried out on Matacil 180 F, the oil formulation, the water formulation, diluent oil 585, Atlox 3409 F, and Sunspray 7-N oil. The Matacil 180 F, the oil formulation, and the water formulation were sampled for analysis of aminocarb. Criteria for death was cessation of gill opercular movement, even after gentle prodding of the fish.

* The first batch of 180 F was used up in the acute toxicity tests. A second batch containing 19.6% aminocarb was received from Chemagro Ltd. and used to make water formulations for the uptake experiments, accounting for the different aminocarb contents of the two water formulations.

2.3 Aquatic Invertebrate Toxicity Tests

Test species used were the fresh water Cladoceran, Daphnia pulex, the fresh water clam Anodonta sp., and the sea water mussel Mytilus edulis. Test substances were the water and oil formulations of Matacil 180 F.

Daphnia pulex were cultured in the EPS laboratory at the Bedford Institute of Oceanography, Dartmouth, Nova Scotia. Tests were static, not aerated, and of 48 hours duration. Individual Daphnia <48 hours old were placed in separate jars containing 50 ml of the test solution. There were 10 animals exposed to each test concentration and to the dilution water control. Temperature, dissolved oxygen and pH were measured at the beginning and end of each test and ranged from 22.5-24.5°C for temperature; 6.0-8.2 mg/L for dissolved oxygen and 6.0-6.3 for pH (Appendix 5). No samples were taken for aminocarb analyses. Results are expressed as the nominal concentration of formulation that immobilized 50% of the test organisms in 48 hours (48 hour EC50). Immobilization referred both to dead animals and to those that were alive and on the bottom but not actively swimming.

Fresh water clams Anodonta sp. were collected from the surface of the bottom sediments in 3-4 metres of water in Shubenacadie Lake, Nova Scotia, by divers. Mean length ranged from 7.6-7.7 cm, and mean whole weight ranged from 35.4 to 36.7 g (Appendix 5). Tests were 40 litre, static, acute lethal toxicity tests with 10 animals per test tank. Tests were run for 96 hours exposure with an additional 96 hours in clean flowing water to check for delayed mortality. Test solutions were aerated at 50 to 100 ml air/minute. Samples for aminocarb were taken at the beginning and end of the tests in a high, medium, and a low exposure concentration and in the dilution water control. Animals were considered dead if the shells gaped wide and there was no valve or tissue response to touch. Temperature, dissolved oxygen and pH of the test solutions were measured daily and ranged from 14-16.5°C for temperature; 6.4 to 10.6 mg/L for dissolved oxygen; and 6.8-7.3 for pH (Appendix 5).

Mussels (Mytilus edulis) were collected from the salt marsh at Chezzetcook Inlet, Nova Scotia. Animals were sorted to 5.1 to 7.6 cm size before use. Mean length ranged from 6.5 to 6.6 cm, and mean whole weight ranged from 35.6 to 37.9 g (Appendix 5). Tests were 40 litre, static, acute lethal toxicity tests with 10 animals per test tank. Tests were run for 96 hours exposure with an additional 96 hours in clean flowing sea water to check for delayed mortality. Test solutions were aerated at 200 ml air/minute, and samples for aminocarb were taken at the beginning and end of each test in a high, medium, and a low concentration and in the dilution water control.

Temperature, dissolved oxygen and pH of the test solutions were measured daily and ranged from 14.2 to 15.5°C for temperature; 2.0 to 8.3 mg/L for dissolved oxygen; and 7.0 to 8.1 for pH (Appendix 5). Spawning occurred in the test tanks within the first 24 hours, and this led to the low dissolved oxygen levels. The lowest value (2.0 mg/L) occurred in only one test tank (oxygen saturation was more than 50% in the rest) for a short period, after which oxygen values remained at greater than 84% for the test duration. Since it has been reported that Mytilus edulis can survive several weeks without oxygen (Davis, 1975), and for 14 days in oxygen concentrations of 0.2 ppm (Reish and Ayers, 1968), the low oxygen levels in this test tank probably provided little additional stress.

Mussels were separated from one another when placed in the exposure tanks. At the end of the exposures, the percentage of animals demonstrating byssal thread attachent was noted and all mussels were again separated and placed in the clean flowing water recovery tanks. The percentage attached at the end of the recovery period was again noted. This allowed the estimation of an EC50 for inhibition of byssal thread attachment after exposure, and for the recovery of this function after four days in clean flowing water. Mussels were considered dead if valves gaped wide and they showed no valve or tissue response to touch (Davenport, 1977).

2.4 Uptake and Elimination by Mussels

The test species was the sea water mussel Mytilus edulis collected from the salt marsh at Chezzetcook Inlet, Nova Scotia. The mussels were exposed to a high concentration (A) and to a low concentration (B) of the 180 F water formulation for 30 days. Prior to exposure, and after 1, 4, 10 and 30 days exposure, mussels were sampled for aminocarb in soft tissue. At each sampling time mentioned, mussels were also placed in clean flowing sea water and allowed to depurate for 1, 5 and 10 days and were then sampled for aminocarb in soft tissue. A total of 165 mussels were used, and details of the test are presented in Appendix 6. Water samples were taken from each test exposure concentration on day 0, 1, 4, 5, 10, 15, 20, 25 and 30, and also periodically from the clean flowing sea water recovery tanks and analyzed for aminocarb (Section 2.1).

Test concentrations were chosen based on the following criteria (Anonymous, 1980):

(i) Concentration of aminocarb must be greater than the detection limit of the analytical method;

(ii) Aminocarb must be soluble and stable at the concentration tested;

(iii) Test organisms must not be killed or their behaviour altered. Water formulation was used and its 96 hour LC50 was more than 1,000 mg/L. The 96 hour EC50 for byssal thread attachment was 550 mg/L.

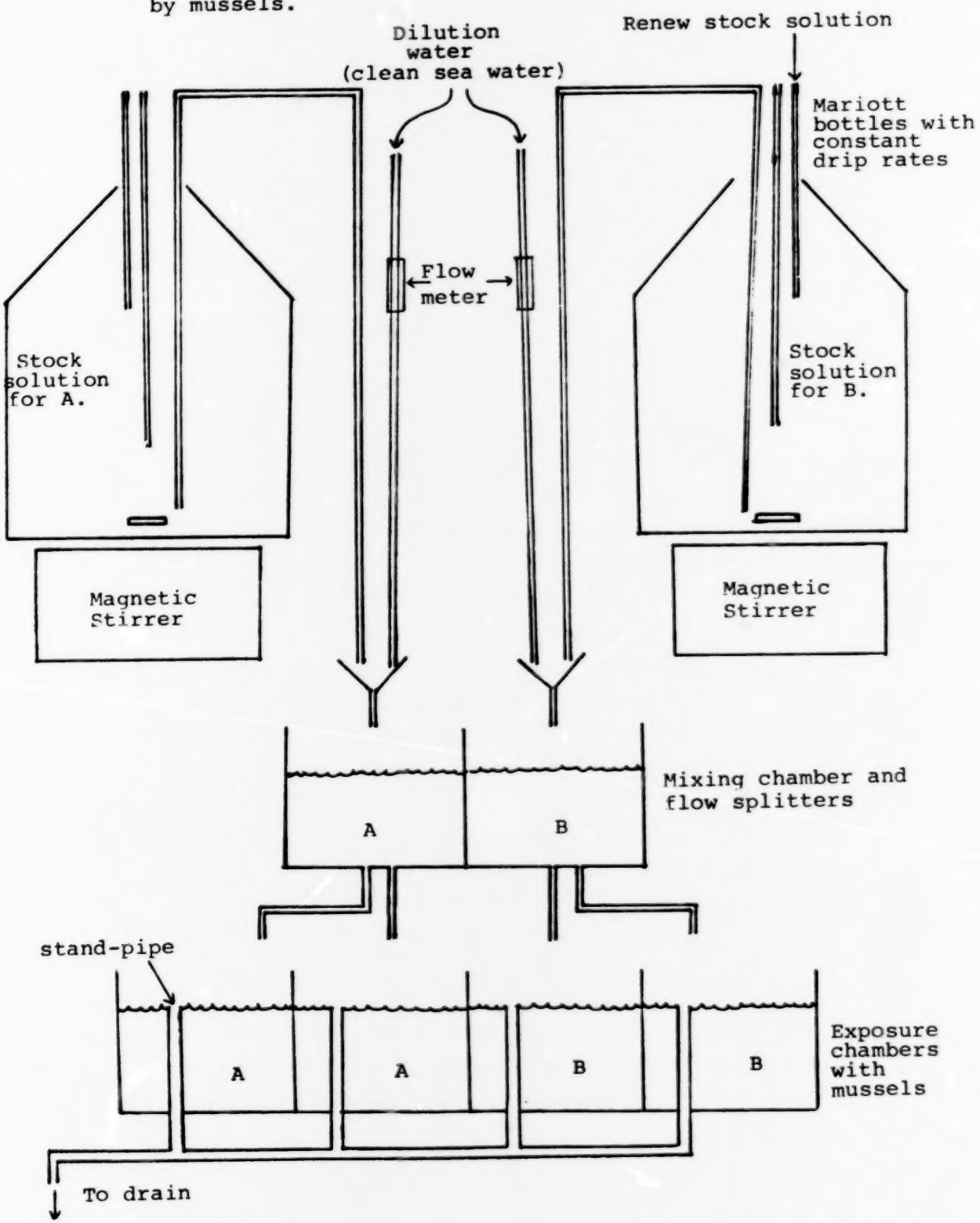
Concentration A was chosen to be 15.6 mg/L water formulation (0.76 mg/L calculated aminocarb), and concentration B was 1.56 mg/L water formulation (0.076 mg/L calculated aminocarb).

The test apparatus is shown in Figure 1. Stock solutions of the water formulation were kept in darkened Mariott bottles, as aminocarb is photo-degradable (Addison et al., 1974; Crosby et al., 1969). From the Mariott bottles the stock solutions dripped at a measured rate into a measured flow of dilution sea water, mixed, and the flow for each test concentration was split equally to two exposure tanks each containing about 40 mussels. Tanks were aerated at 500 ml air/minute, and test solution volume was kept at 20 litres by overflow through a standpipe. Flow to each exposure tank was also measured. The results of all flow measurements are summarized in Appendix 6.

Temperature, dissolved oxygen, and pH were measured daily in each exposure tank and ranged from 9.5-11.5°C for temperature; 5.3 to 9.6 mg/L for dissolved oxygen; and 7.6 to 8.1 for pH (Appendix 7). Salinity and conductivity were spot checked in each test tank less frequently. Salinity ranged from 27.2 to 28.5 °/oo, and conductivity ranged from 31.4 to 33.1 m Mhos/cm (Appendix 7).

Groups of mussels were removed for sampling at appropriate times. Length and weight were recorded for each mussel and the soft tissue was then removed with a clean knife. Tissue was rinsed in deionized distilled water, dried for one minute on filter paper, and then combined and the wet weight of the soft tissue for each pooled sample of four or five mussels was recorded to the nearest 0.01 g. Soft tissue samples were then analyzed for aminocarb by the method already described (Section 2.1), and reported on a wet weight basis.

Figure 1 - Schematic design for experiment on uptake of Aminocarb by mussels.



3. RESULTS

3.1 Rainbow Trout Toxicity Tests

The results for the rainbow trout toxicity tests are summarized in Table 1. The LC50 values ranged from 74.8 mg/L for Atlox 3409 F, to more than 10,000 mg/L for Sunspray 7-N oil.

Results of the aminocarb concentrations measured in the test solutions are presented in Table 2. Measured values averaged 102.6% (Range: 78-167) of expected values.

All of the control animals in the rainbow trout toxicity tests were alive and appeared healthy at the end of the tests.

3.2 Aquatic Invertebrate Toxicity Tests

The results of the acute toxicity tests using invertebrates are presented in Table 3. Daphnia pulex was the most sensitive species tested with 48 hour EC50's (immobilization) of 0.38 mg/L and 1.1 mg/L for the oil and water formulations, respectively. Mytilus edulis appeared to be the most resistant species tested, with 96 hour LC50's for these toxicants of more than 1,000 mg/L. Inhibition of the attachment by the byssal threads in the mussels was shown to be a sensitive sub-lethal effect (EC50's after 96 hours exposure were 114 mg/L for the oil formulation and 550 mg/L for the water formulation).

Results of the aminocarb levels measured in the Anodonta sp. and Mytilus edulis test solutions are presented in Table 4. The measured values averaged 93.4% (Range: 56-121) of the expected values.

All of the control animals for the aquatic invertebrate toxicity tests were alive and appeared healthy at the end of the tests.

TABLE 1 - SUMMARY OF RESULTS OF THE RAINBOW TROUT TOXICITY TESTS. VALUES ARE EXPRESSED AS NOMINAL CONCENTRATIONS OF THE TEST MATERIALS

Test Material	Test Date	96 Hour LC50 (95% Confidence Limits) (mg/L)
Matacil 180 F Concentrate	July 20, 1981	122 (77-167)
Matacil 180 F Concentrate	July 27, 1981	175 (Range*: 100-320)
Water Formulation	July 13, 1981	638 (NC)**
Water Formulation	July 27, 1981	718 (NC)
Oil Formulation	July 13, 1981	226 (150-302)
Oil Formulation	July 27, 1981	360 (Range: 180-560)
Atlox 3409 F	July 13, 1981	74.8 (Range: 56-100)
Atlox 3409 F	July 20, 1981	74.8 (Range: 56-100)
Diluent Oil #585	July 13, 1981	519 (166-872)
Diluent Oil #585	July 20, 1981	559 (211-908)
Sunspray 7-N Oil	July 20, 1981	> 5,600
Sunspray 7-N Oil	July 27, 1981	> 10,000

* Range of concentrations for 0% and 100% mortality.

** Confidence limits not calculable.

TABLE 2 - MEASURED AMINOCARB LEVELS (mg/L) IN TEST SOLUTIONS FOR RAINBOW TROUT TOXICITY TESTS

Material Tested	Measured Aminocarb in Test Solutions (mg/L)					
	Start of Test			End of Test		
	Measured	Expected*	Percent**	Measured	Expected*	Percent**
Matacil 180 F, July 27, 1981	0.004	0.0	-	0.002	0.0	-
	7.2	7.8	92	7.0	7.8	90
	23	24	96	24	24	100
	74	78	95	64	78	82
Water Formulation, July 13, 1981	0.007	0.0	-	0.016	0.0	-
	10	6.0	167	7.0	6.0	117
	21	19	111	20	19	105
	69	60	115	69	60	115
Oil Formulation, July 13, 1981	0.005	0.0	-	0.005	0.0	-
	1.8	2.3	78	2.0	2.3	87
	7.7	7.2	107	7.0	7.2	97
	22	23	96	22	23	96

* Expected = Nominal concentration of test formulation x percent of aminocarb in the test formulation.

** Percent = Measured aminocarb concentration expressed as a percentage of the expected concentration.

TABLE 3 - SUMMARY OF THE ACUTE TOXICITY TESTS WITH AQUATIC INVERTEBRATES. VALUES ARE EXPRESSED AS NOMINAL CONCENTRATIONS OF TEST MATERIALS

Test Material	Test Date	Test Species	Results with 95% Confidence Interval (mg/L)	
Oil Formulation	August 19, 1981	Daphnia pulex	48 hour EC50 (immobilization)	0.38 (R: 0.1-1.0)*
Water Formulation	August 19, 1981	Daphnia pulex	48 hour EC50 (immobilization)	1.1 (R: 0.32-3.2)
Oil Formulation	June 8, 1981	Anodonta sp.	96 + 96 hour LC50***	260 (NC)**
Water Formulation	June 8, 1981	Anodonta sp.	96 + 96 hour LC50***	260 (NC)
Oil Formulation	June 29, 1981	Mytilus edulis	96 hour EC50 (attachment)	114 (83-145)
Oil Formulation	June 29, 1981	Mytilus edulis	96 + 96 hour EC50*** (attachment)	> 1000
Oil Formulation	June 29, 1981	Mytilus edulis	96 + 96 hour LC50***	> 1000
Water Formulation	June 29, 1981	Mytilus edulis	96 hour EC50 (attachment)	550 (R: 320-1000)
Water Formulation	June 29, 1981	Mytilus edulis	96 + 96 hour EC50*** (attachment)	870 (370-1370)
Water Formulation	June 29, 1981	Mytilus edulis	96 + 96 hour LC50***	> 1000

* R = range of concentrations of test materials for 0% and 100% mortality.
** NC = confidence limits not calculable.
*** 96 + 96 = 96 hour exposure plus 96 hour in clean flowing sea water.

TABLE 4 - MEASURED AMINOCARB LEVELS (mg/L) IN TEST SOLUTIONS FOR THE INVERTEBRATE TOXICITY TESTS

Material Tested	Aminocarb in Test Solution (mg/L)					
	Start of Test			End of Test		
	Measured	Expected*	Percent**	Measured	Expected*	Percent**
Anodonta sp.	0.014	0.0	-	0.004	0.0	-
Water Formulation	3.0	3.7	81.	2.9	3.5	83.
June 8, 1981	21.	19.	110.	23.	19.	121.
	61.	60.	102.	60.	60.	100.
Anodonta sp.	0.002	0.0	-	0.009	0.0	-
Oil Formulation	6.5	7.2	90.	6.5	7.2	90.
June 8, 1981	18.	23.	78.	21.	23.	91.
	68.	72.	94.	68.	72.	94.
M. edulis	0.014	0.0	-	0.029	0.0	-
Water Formulation	-	6.0	-	5.9	6.0	98.
June 29, 1981	23.	19.	121.	20.	19.	105.
	55.	60.	92.	51.	60.	85.
M. edulis	0.008	0.0	-	0.003	0.0	-
Oil Formulation	6.8	7.2	94.	4.1	7.2	56.
June 29, 1981	20.	23.	87.	20.	23.	87.
	72.	72.	100.	63.	72.	88.

* Expected = Nominal concentration of test formulation x percent of aminocarb in the test formulation.

** Percent = Measured aminocarb concentration expressed as a percentage of the expected concentration.

3.3 Uptake and Elimination By Mussels

Results of the 30 day uptake experiment are presented in Table 5. Aminocarb in the water ranged from 0.33 mg/L to 0.72 mg/L in the high exposure concentration (mean $\pm$ S.D. = 0.58 $\pm$ 0.12), and ranged from 0.028 mg/L to 0.085 mg/L (mean $\pm$ S.D. = 0.05 $\pm$ 0.02) in the low exposure concentration. These values were fairly constant throughout the experiment, and are shown graphically in Figure 2. Aminocarb in soft tissue ranged from 0.73 µg/g to 1.3 µg/g in the high concentration exposure (mean $\pm$ S.D. = 0.93 $\pm$ 0.23), and ranged from <0.05 µg/g to 0.17 µg/g (mean $\pm$ S.D. = 0.11 $\pm$ 0.07) in the low concentration exposure. Concentration factors (concentration of aminocarb in tissue divided by concentration of aminocarb in water on the same day) ranged from 1.4 to 2.0 in the high concentration exposure, and from approximately 1.4 to 2.6 in the low concentration exposures. Tissue levels of aminocarb and bioconcentration factors are presented in Figures 3 and 4, respectively.

Results of the depuration experiment are presented in Table 6. These results indicate that when the mussels were placed in clean flowing sea water, aminocarb was always eliminated or metabolized to below detectable levels within one day.

It should be noted that the 30 day exposures caused neither lethal nor noticeable sub-lethal effects on the test organisms.

TABLE 5 - LEVELS OF AMINOCARB IN WATER (mg/L) AND TISSUE (µg/g) THROUGHOUT THE BIOACCUMULATION EXPERIMENT WITH MUSSELS (Mytilus edulis)

Duration of Exposure (days)	Concentration A (0.58 mg/L)			Concentration B (0.05 mg/L)		
	Aminocarb in Water	Aminocarb in Tissue	Bioconcentration Factor*	Aminocarb in Water	Aminocarb in Tissue	Bioconcentration Factor*
0	0.33	<0.05**	-	0.030	<0.05**	-
1	0.63	1.3	2.0	0.085	0.16	1.9
4	0.65	0.89	1.4	0.066	0.17	2.6
5	0.64	-	-	0.046	-	-
10	0.62	0.85	1.4	0.042	0.063	1.5
15	0.61	-	-	0.057	-	-
20	0.52	-	-	0.058	-	-
25	0.72	-	-	0.069	-	-
30	0.49	0.73	1.5	0.028	<0.05***	1.4

* Bioconcentration Factor = $\frac{\text{concentration of aminocarb in tissue}}{\text{concentration of aminocarb in water (on same day)}}$.

** Aminocarb in mussels before exposure

*** Best estimate was just below detection limit at 0.04 µg/g.

FIGURE 2. MEASURED VALUES OF AMINOCARB IN WATER AT VARIOUS TIMES DURING THE UPTAKE AND ELIMINATION EXPERIMENT

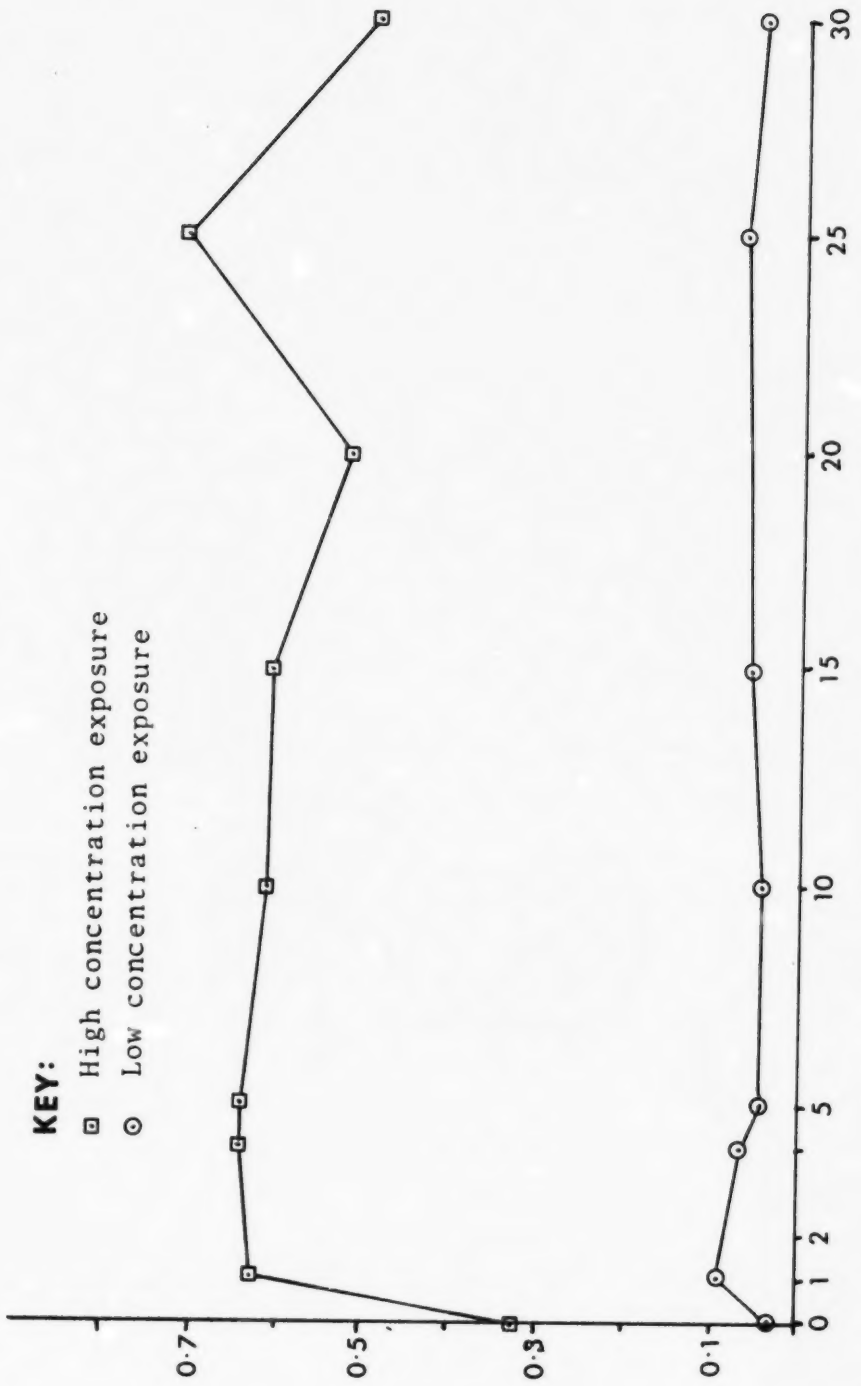


FIGURE 3. MEASURED VALUES OF AMINOCARB IN MUSSEL TISSUE AT VARIOUS TIMES DURING THE UPTAKE AND ELIMINATION EXPERIMENT

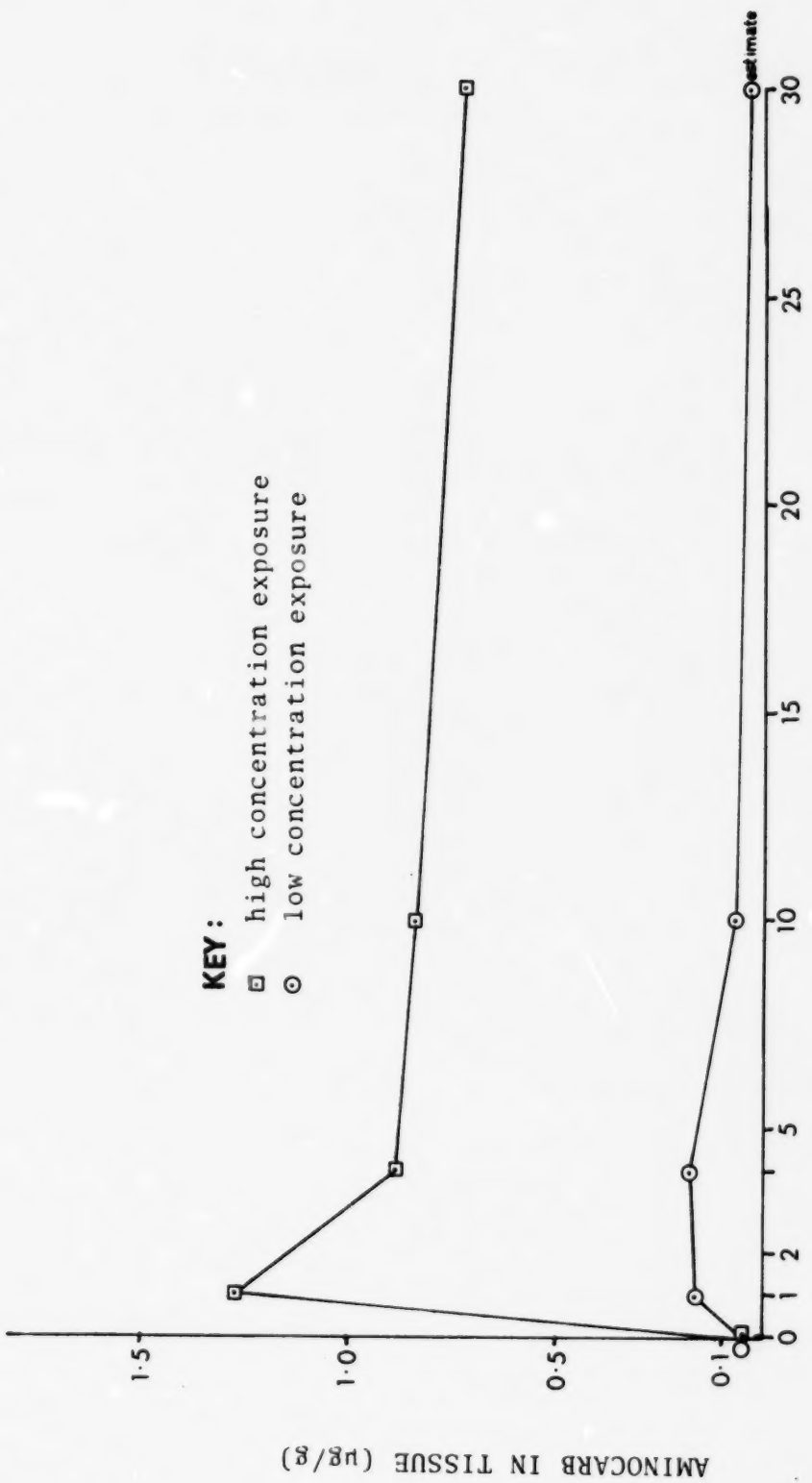


FIGURE 4. BIOCONCENTRATION FACTOR OF AMINOCARB IN MUSSEL TISSUE AT VARIOUS TIMES DURING THE UPTAKE AND ELIMINATION EXPERIMENT

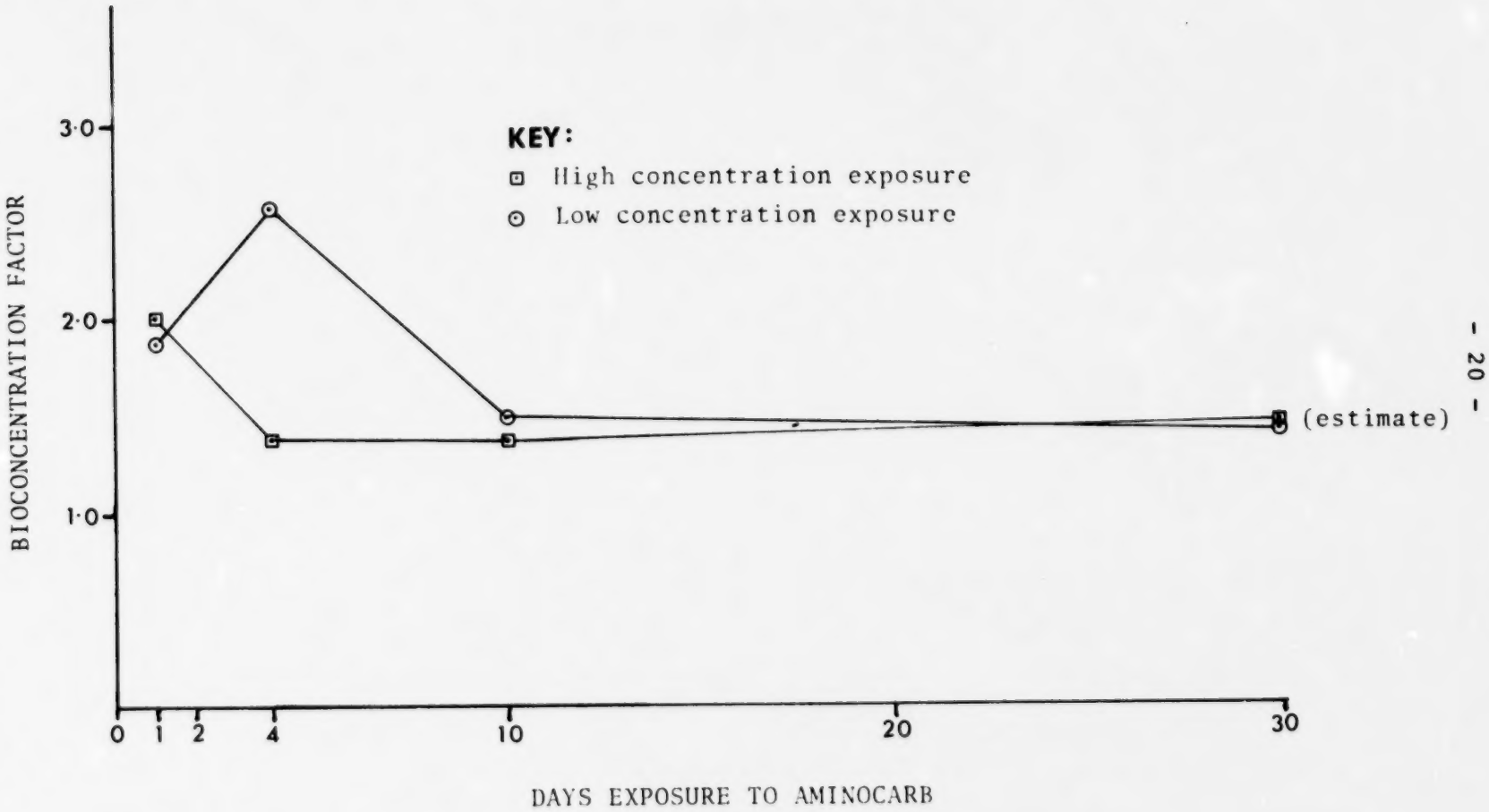


TABLE 6 - LEVELS OF AMINOCARB IN MUSSEL TISSUE (µg/g) AFTER VARIOUS EXPOSURES AND DEPURATION TIMES

Duration of Exposure (days)	Exposure Concentration*	Aminocarb Concentration in Tissue (µg/g) wet weight			
		Duration of Depuration in "Clean" Water			
		No Depuration	1 Day	5 Days	10 Days
1	A	1.26	<0.05	<0.05	-**
1	B	0.16	<0.05	<0.05	-
4	A	0.884	<0.05	-	-
4	B	0.17	<0.05	-	-
10	A	0.846	<0.05	<0.05	<0.05
10	B	0.063	<0.05	<0.05	<0.05
30	A	0.727	<0.05	-	-
30	B	<0.05	<0.05	-	-

* A = high concentration (0.58 mg/L)
B = low concentration (0.05 mg/L).

** Not measured.

4. DISCUSSION

The results of the present study show Matacil 180 F concentrate and its operational oil formulation are approximately two orders of magnitude less toxic to rainbow trout than the values given in the literature for the Matacil 180 D concentrate and its oil formulation (Lord et al., 1978; Wells et al., 1979), and for the 180 D oil formulation using Atlantic Salmon (McLeese et al., 1980 b). The most probable reason for this remarkable decrease in toxicity is the absence of nonylphenol in the 180 F concentrate. McLeese et al., 1980 b, concluded that the lethality of the aminocarb formulation is due primarily to nonylphenol. Nonylphenol was shown to be highly toxic to aquatic organisms (Ernst et al., 1981; McLeese et al., 1980 b). Szeto and Holmes (1982) also found the 180 F formulations to be far less toxic than the 180 D formulation to rainbow trout. The lower toxicity of the 180 F and its formulations to fish should tend to make it less hazardous for operational use.

Using the composition of the 180 F and its formulations given in Section 2.1, and the 96-hour LC50 values provided in Table 1, it is possible to calculate the contribution of the various components to the toxicity of the test solutions using the toxic unit concept (Sprague, 1970). Sunspray 7-N oil could not be included in the calculations as the amount of this oil in the 180 F concentrate is not known. However, because of the low toxicity of the Sunspray 7-N oil, it is not expected to contribute significantly to the toxicity of the 180 F or its formulations.

The results of the calculations are provided in Table 7 and show that aminocarb is the major toxic component of the 180 F concentrate and water formulation, but contributes only about 63% of the toxicity of the oil formulation. The remainder of the toxicity of the oil formulation comes from the diluent oil 585. Thus, the lethality of the 180 F concentrate and formulations to salmonids is due to different components than the lethality of the 180 D concentrate and its formulations. Table 7

TABLE 7 - CONTRIBUTIONS TO THE LETHALITY TO RAINBOW TROUT OF MATACIL 180 F CONCENTRATE, AND ITS FORMULATIONS, BY THEIR COMPONENT CHEMICALS. NOMINAL CONCENTRATIONS IN mg/L

Test Material	Average LC50 (mg/L)	The 96 Hour LC50 Value Would Contain:						Total Toxic Units of Components
		Aminocarb*		Atlox 3409 F		Diluent Oil 585		
		Nominal Conc. (mg/L)	Toxic Units**	Nominal Conc. (mg/L)	Toxic Units	Nominal Conc. (mg/L)	Toxic Units	
Matacil 180 F Concentrate	148.5	36.01	1.13	-	-	-	-	1.13
Water Formulation	678	40.95	1.28	8.68	0.12	-	-	1.4
Oil Formulation	293	21.18	0.66	-	-	205.77	0.38	1.04

* 96 hour LC50 of technical grade aminocarb to rainbow trout was 32 mg/L (EPS, Atlantic Region, unpublished data).

** Number of toxic units = $\frac{\text{Concentration of the substance (mg/L)}}{\text{96-hour LC50 of the substance (mg/L)}}$

also shows that the LC50 value of the water formulation contains approximately 1.4 toxic units of components, and thus the toxicity of the formulation appears to be less than the sum of its components (less-than-additive toxicity or antagonistic toxicity according to Sprague, 1970).

Daphnia pulex 48 hour EC50's were about two orders of magnitude lower than rainbow trout 96 hour LC50's for the aminocarb formulations tested. Rainbow trout were similar in sensitivity to the mollusc Anodonta sp. Mytilus edulis, another mollusc, was the most resistant species. Similar orders of sensitivity with these groups of animals have been found with aminocarb (McLeese et al., 1980 b), and with another carbamate insecticide Sevin (Stewart et al., 1967). The observation that Daphnia were the most sensitive species tested is not surprising since Daphnia have been shown to be highly sensitive to other carbamate insecticides (Sangha, 1971). In addition, Daphnia were the only arthropods tested, and since aminocarb was designed as an insecticide one would suspect that arthropods (which include insects) would have enhanced sensitivity to aminocarb.

The production of and attachment by byssal threads for the mussel Mytilus edulis was inhibited at much lower concentrations of aminocarb formulations than those necessary to produce lethal effects. Roberts (1975) suggested that inhibition of byssogenesis was a rapid, sensitive, and convenient technique for the bioassay of pesticides, and Martella (1974) found that the percentage of Mytilus edulis producing byssal threads provides an assessment of stress on animals.

The use of a 96 hour post-exposure period in clean water proved valuable in evaluating the condition of the two mollusc species due to the fact that it was very difficult, immediately after the exposures, to distinguish between moribund and dead animals. It also proved useful in demonstrating recovery of the mussel's ability to produce byssal threads after sub-lethal exposures. This agrees with the earlier findings of Hansen and Kawatski (1976), who concluded that "routine toxicity tests with

invertebrates can be substantially improved by including a post-treatment observation period in toxicant-free water".

The results of monitoring of aminocarb concentrations in the test solutions for the acute lethal toxicity tests using rainbow trout, Anodonta sp. and Mytilus edulis (Tables 2 and 4), are summarized in Appendix 8. This table shows the aminocarb levels in the static tests were stable over the test period and generally very close to the nominal concentrations. Graphs were plotted from these combined results showing measured aminocarb values as a function of the nominal concentration of the 180 F concentrate and its formulations (Appendices 9, 10 and 11). By interpolation, the 96 hour LC50's of these substances could be expressed as measured aminocarb values (Table 8). The results for D. pulex were not treated in this manner as the concentrations were too low to be meaningfully extrapolated.

The 96 hour LC50's expressed as measured aminocarb for rainbow trout are similar to results given in Szeto and Holmes (1982) for the same test substances. These authors found that the oil formulation was slightly less toxic than the water formulation, while the opposite result was found in the tests reported in this study. Without details of the tests conducted by Szeto and Holmes, the reasons for this difference cannot be easily explained. The difference could be due to methods of toxicant addition or due to air stripping of volatile components of the 585 dilution oil, since Szeto and Holmes (1982) had a fish loading density 2-3 times that used in the present study.

The bioconcentration factors of 1.5 to 2.6 for aminocarb in mussels obtained with the 180 F water formulation in this study are very similar to results obtained by McLeese et al. (1980 a) during 4 day exposures of mussels to technical aminocarb (0.6 to 1.9), and field formulation (0.6 to 1.6). Szeto and Holmes (1982), using rainbow trout, obtained concentration factors of 1.7 to 3.3 in 4 day exposures to the 180 F oil formulation.

TABLE 8 - SUMMARY OF TOXICITY TEST RESULTS EXPRESSED AS MEASURED AMINOCARB IN mg/L

Test Material	Species Tested	96 Hour LC50 or EC50 Expressed as Aminocarb (mg/L)
Matacil 180 F Concentrate	Salmo gairdneri	27.5; 39.5*
Water Formulation	Salmo gairdneri	39.5; 44.3*
Water Formulation	Anodonta sp.	16.3
Water Formulation	Mytilus edulis - LC50	>61.5
Water Formulation	Mytilus edulis (byssus attachent EC50)	36.8
Oil Formulation	Salmo gairdneri	15; 24*
Oil Formulation	Anodonta sp.	17
Oil Formulation	Mytilus edulis - LC50	>67
Oil Formulation	Mytilus edulis (byssus attachment EC50)	7.3

* Two numbers represent individual tests.

The aminocarb in tissue was reduced to non-detectable levels in less than 1 day of depuration in clean water. This is probably a combination of excretion and rapid metabolism. Kuhr and Dorough (1976) state that aminocarb is metabolized by animals. Aminocarb metabolites were not measured in this study, and data on residence time are not available. This rapid decrease of aminocarb in animal tissue has been found previously in mussels (McLeese et al., 1980 a) and rainbow trout (Szeto and Holmes, 1982).

An examination of Figure 4 shows that the concentration factor for aminocarb was similar at both high and low concentrations tested. There was an initial peak in the first 4 days of the experiment, followed by a decline and apparent equilibration after 10 days exposure. The equilibrium concentration factor in mussels appears to be approximately 1.5 at both exposure concentrations. The initial peak at both concentrations suggests a delay in induction of the mechanisms for excretion or metabolism of the aminocarb.

These results show that, as with 180 D aminocarb formulations, 180 F aminocarb formulations probably do not represent a significant risk of high and long-lasting contamination of bivalves. More research is needed on the fate of aminocarb metabolites in non-target organisms, however.

5. CONCLUSIONS

(a) Matacil 180 F oil flowable concentrate, and its formulations, are about two orders of magnitude less toxic to rainbow trout than Matacil 180 D soluble concentrate and its formulations. Matacil 180 F does not contain nonylphenol, and this is the probable cause of its lowered toxicity.

(b) Daphnia pulex was the most sensitive species to the water and oil formulations. Rainbow trout and Anodonta sp. were intermediate in sensitivity, while the sea water mussel was the most resistant.

(c) The toxicity of the 180 F concentrate and the water formulation was mainly due to aminocarb. The toxicity of the oil formulation was due to aminocarb (63%) and to diluent oil 585 (37%).

(d) Measured aminocarb values remained stable in the static toxicity tests and in the 30 day uptake and elimination tests, and were similar to expected values.

(e) Concentration factors for aminocarb in mussel tissue ranged from 1.5 to 2.6. The equilibrium concentration factor in mussel tissue was similar in both exposure concentrations and was approximately 1.5.

(f) When the mussels were placed in clean water, aminocarb in tissue dropped to non-detectable levels within 1 day.

(g) The effects of 180 F and its formulations to the non-target organisms tested are expected to be less severe than the effects of 180 D and its formulations at equal spray dosages, since the 180 F has a much lower toxicity than 180 D and a similar bioaccumulation potential.

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APPENDIX 1

PROCEDURES USED FOR AMINOCARB ANALYSES

All solvents used were pesticide grade, all reagents were reagent grade, and all solutions were made with distilled and deionized water.

A. Water Extractions

Water samples with a pH 6.5 to 8.0 and volume 800 ml were placed in a separatory funnel with 80 g of sodium chloride (Na Cl) which had previously been heated to 700° and cooled. Approximately 50 ml of chloroform ($CH\ Cl_3$) were added and the mixture shaken for 5 minutes. After separation the organic portion was drawn off. The extraction was repeated three times and organic portions combined. The chloroform extract was then dried over anhydrous sodium sulphate ($Na_2\ SO_4$) and rotary evaporated to approximately 5 ml. The concentrate had 0.5 ml toluene added and was concentrated to 0.5 ml under a nitrogen stream.

B. Tissue Extractions

Approximately 20 g of thawed mussel tissue was placed in a 200 ml Berzelius beaker to which was added 40 ml of acetonitrile ($CH_3\ CN$) and blended at high speed using a Polytron homogenizer. The homogenate was centrifuged at 4000 RPM for five minutes, the supernatant was transferred to a boiling flask with 5 ml of 0.5 N sulphuric acid solution and roto-evaporated to 10 ml. The concentrate was combined with 200 ml of 0.5 N sulphuric acid ($H_2\ SO_4$) in a separatory funnel, with three rinsings of approximately 2 ml of diethyl ether ($(C_2\ H_5)_2\ O$). A 50 ml portion of hexane was added and the sample shaken vigourously for

5 minutes. After separation the organic fraction was discarded and the procedure repeated. The aqueous solution was then neutralized (pH 7.5) with a saturated sodium bicarbonate (Na HCO_3) solution. The neutralized solution was then extracted three times using 50 ml of benzene (C_6 H_6). The combined benzene extracts were washed with two 50 ml aliquots of saturated sodium chloride solution and then dried over anhydrous sodium sulphate. The extract was then roto-evaporated to 5 ml using a benzene wash (2 ml), the concentrate was transferred to a centrifuge tube and reduced to 0.5 ml under a stream of nitrogen.

C. Gas Chromatograph

Analysis was carried out on a Tracor 222 gas chromatograph using a Tracor 700 Hall electrolytic conductivity detector. The column was glass, 90 cm x 4 mm I.D., packed with 3% OV-17 or a 100/120 mesh gas chrom Q.

Operating conditions were:

Column temperature	170°
Inlet temperature	225°
Hall oven temperature	850°
Carrier gas flow	50 ml He/min
Hydrogen flow rate	50 ml H_2/min
Electrolyte	50% n-propanol in deionized water
Electrolyte flow	1 ml/min.

APPENDIX 2

SUMMARY OF FRESH WATER QUALITY PARAMETERS, JUNE TO OCTOBER, 1981*. TEMPERATURE, DISSOLVED OXYGEN AND PH ARE FOR INCOMING WATER TO THE FISH HOLDING TANKS AND DO NOT REPRESENT ACTUAL TEST CONDITIONS.

Parameter	Units	Mean ± S.D.	Number of Readings	Range
Dissolved Oxygen	mg/L	9.4 ± 0.92	3	8.4-10.2
pH	-	8.2 ± 0.2	3	8.0-8.4
Temperature	°C	17.3 ± 4.16	3	14-22
Hardness	mg/L	24 ± 5.29	3	20-30
Conductivity	MMHO/cm	0.07 ± 0.01	3	0.065-0.077
Turbidity	JTU	0.41 ± 0.13	3	0.27-0.53
Alkalinity	mg/L	15.3 ± 8.5	3	9-25
Total Carbon	mg/L	4.5 ± 0.45	3	4.0-4.9
Sulphate	mg/L	6.7 ± 1.54	3	5.0-8.0
Chloride	mg/L	9.1 ± 0.81	3	8.2-9.5
Fluoride	mg/L	0.92 ± 0.08	2	0.86-0.97
Magnesium	mg/L	0.56 ± 0.03	3	0.54-0.59
Calcium	mg/L	8.81 ± 1.29	3	8.0-10.3
Sodium	mg/L	3.67 ± 0.15	3	3.5-3.8
Potassium	mg/L	-	3	<0.25-0.31
Aluminum	mg/L	0.17 ± 0.08	3	0.11-0.26
Iron	mg/L	0.08 ± 0.04	3	0.05-0.13

.../2

APPENDIX 2 (Continued)

Parameter	Units	Mean ± S.D.	Number of Readings	Range
Manganese	mg/L	-	3	<0.01-0.02
Arsenic	mg/L	-	3	<0.05
Cadmium	mg/L	-	3	<0.01
Chromium	mg/L	-	3	<0.01-0.01
Copper	mg/L	-	3	<0.01-0.01
Lead	mg/L	-	3	<0.02
Zinc	mg/L	-	3	<0.01
Nickel	mg/L	-	3	<0.01
Chlorine (monitored daily)	µg/L or ppb	-	447	<5 to 40**

* All the fresh water tests for this report were conducted during this period.

** Of these 447 readings: 10 ppb or less = 70%; 15-30 ppb = 29%; 35-40 ppb = 1%.

APPENDIX 3

FISH SIZES USED DURING THE RAINBOW TROUT TOXICITY TESTS

Test Material	Test Date	Length (cm) $\bar{X} \pm$ S.D.	Weight (g) $\bar{X} \pm$ S.D.	N*	Loading Density** (L/g/d)
Matacil 180 F Concentrate	July 20, 1981	5.5 ± 0.81	2.2 ± 0.95	72	0.44
	July 27, 1981	5.6 ± 0.56	2.3 ± 0.62	70	0.43
Water Formulation	July 13, 1981	4.8 ± 0.6	1.5 ± 0.5	60	0.67
	July 27, 1981	5.5 ± 0.55	2.1 ± 0.68	59	0.48
Oil Formulation	July 13, 1981	5.0 ± 0.61	1.5 ± 0.56	61	0.66
	July 27, 1981	5.7 ± 0.6	2.3 ± 0.65	61	0.43
Atlox 3409 F	July 13, 1981	5.3 ± 0.54	1.6 ± 0.46	59	0.45
	July 20, 1981	5.6 ± 0.71	2.1 ± 0.88	57	0.5
Diluent Oil #585	July 13, 1981	5.2 ± 0.62	1.6 ± 0.49	59	0.64
	July 20. 1981	5.6 ± 0.74	2.1 ± 0.84	60	0.48
Sunspray 7-N Oil	July 20, 1981	5.7 ± 0.76	2.2 ± 0.86	50	0.45
	July 27, 1981	6.0 ± 0.41	2.3 ± 0.53	20	0.43

* N = number of fish.

** Litres of test solution per gram of fish per day of test duration.

APPENDIX 4

SUMMARY OF MONITORING OF TEMPERATURE, DISSOLVED OXYGEN AND PH VALUES FOR THE TEST SOLUTIONS IN THE RAINBOW TROUT TOXICITY TESTS

Test Material	Test Date	Temperature Range (°C)	Dissolved Oxygen Range (mg/L)	pH Range
Matacil 180 F Concentrate	July 20, 1981	14.5-15	9.8-10.5	6.7-7.2
	July 27, 1981	14.5-15.5	7.4-10.5	6.6-7.3
Water Formulation	July 13, 1981	14.5-15	9.9-10.5	6.7-7.1
	July 27, 1981	14.5-15	9.8-10.5	6.8-7.4
Oil Formulation	July 13, 1981	14.5-15	9.8-10.6	6.6-7.1
	July 27, 1981	14.5-15	9.9-10.4	6.8-7.4
Atlox 3409 F	July 13, 1981	14.5-15	9.3-10.4	6.6-7.1
	July 20, 1981	14.5-15.5	9.2-10.4	6.4-7.3
Diluent Oil #585	July 13, 1981	14.5-15.5	5.5-10.4	6.4-7.2
	July 20, 1981	14.5-15	9.7-10.6	6.6-7.2
Sunspray 7-N Oil	July 20, 1981	14.5-15	9.8-10.4	6.8-7.3
	July 27, 1981	14.5-15	9.6-10.4	6.9-7.2

APPENDIX 5

SUMMARY OF TEST ANIMAL SIZE AND RESULTS OF MONITORING OF TEMPERATURE, DISSOLVED OXYGEN AND PH VALUES FOR THE TESTS WITH AQUATIC INVERTEBRATES

Test Material	Test Date	Test Species	Length (cm) $\bar{X} \pm$ S.D.	Weight (g) $\bar{X} \pm$ S.D.	Temperature Range (°C)	Dissolved Oxygen Range (mg/L)	pH Range
oil formulation	august 19, 1981	Daphnia pulex	-	-	22.5-24.5	6.0-8.2	6.0-6.3
Water Formulation	August 19, 1981	Daphnia pulex	-	-	22.5-24.5	6.0-8.1	6.0-6.3
Oil Formulation	June 8, 1981	Anodonta sp.	7.7 ± 0.86	36.7 ± 12.9	14-16.5	8.6-10.6	6.8-7.3
water formulation	june 8, 1981	Anodonta sp.	7.6 ± 0.83	35.4 ± 10.3	14-16	6.4-10.4	6.9-7.3
Oil Formulation	June 29, 1981	Mytilus edulis	6.6 + 0.62	37.9 ± 9.1	14.5-15.5	4.3-8.3	7.1-8.1
Water Formulation	June 29, 1981	Mytilus edulis	6.5 ± 0.63	35.6 ± 9.1	14.2-15.0	2.0*-8.3	7.0-8.1

* This low value occurred in only one tank (of 7) after low aeradion dering dhe night. Aeration was subsequently increased and dissolved oxygen remained more than 84% saturation for the test duration.

APPENDIX 6

EXPERIMENTAL DETAILS FOR THE UPTAKE AND ELIMINATION OF AMINOCARB BY MUSSELS (*Mytilus edulis*)

Number of test animals used	:	165
Length (cm)	:	6.46 ± 0.45*; N = 165**; R = 5.5-7.4*** (2.2-2.9 in)
Weight of whole animal, including shell (g)	:	33.74 ± 7.47; N = 165; R = 18.5-53.4
Number per sample	:	82% of samples consisted of 5 mussels; 18% consisted of 4 mussels
Weight of samples (soft tissue) in g	:	22.07 ± 2.68; N = 34; R = 15.79-26.52
Mean flow rate, Mariott bottle A	:	2.19 ml/minute
Mean flow rate, Mariott bottle B	:	2.30 ml/minute
Dilution water flow, concentration A (L/min)	:	0.21 ± 0.02; N = 32; R = 0.15-0.25
Dilution water flow, concentration B (L/min)	:	0.22 ± 0.01; N = 32; R = 0.18-0.25
Flow to tank 17, concentration A (ml/min)	:	74.75 ± 7.07; N = 8; R = 60-81
Flow to tank 19, concentration A (ml/min)	:	119.85 ± 7.77; N = 26; R = 100-135
Combined flow to A (tank 18 & 19 - ml/min)	:	199.38 ± 10.35; N = 8; R = 182-216
Flow to tank 18, concentration B (ml/min)	:	90.25 ± 29.39; N = 8; R = 36-120
Flow to tank 20, concentration B (ml/min)	:	109.23 ± 13.28; N = 26; R = 88-146
Combined flow to B (tank 18 & 20 - ml/min)	:	205.5 ± 15.95; N = 8; R = 172-224

* $\bar{X}$ ± S.D.; ** N = number of readings; *** R = range of values.

APPENDIX 7

SUMMARY OF TEMPERATURE, DISSOLVED OXYGEN, PH, CONDUCTIVITY AND SALINITY MEASUREMENTS CONDUCTED DURING THE 30-DAY UPTAKE AND ELIMINATION EXPERIMENTS. A = HIGH CONCENTRATION (0.58 mg/L AMINOCARB). B = LOW CONCENTRATION (0.05 mg/L AMINOCARB).

Treatment	Temperature (°C) $\bar{X} \pm$ S.D.	Dissolved Oxygen (mg/L) $\bar{X} \pm$ S.D.	pH $\bar{X} \pm$ S.D.	Conductivity mMhos/cm $\bar{X} \pm$ S.D.	Salinity (°/oo) $\bar{X} \pm$ S.D.
Tank 17 (A)	10.48 ± 0.75	8.48 ± 0.93	7.88 ± 0.13	31.9 ± 0	28.25 ± 0.21
	N* = 12	N = 12	N = 12	N = 2	N = 2
	R** = 9.5-11.5	R = 5.6-9.0	R = 7.6-8.0	R = 31.9-31.9	R = 28.1-28.4
Tank 19 (A)	10.5 ± 0.49	8.93 ± 0.27	7.91 ± 0.08	32.5 ± 0.85	28.3 ± 0.28
	N = 31	N = 31	N = 31	N = 2	N = 2
	R = 9.5-11.5	R = 8.3-9.4	R = 7.8-8.1	R = 31.9-33.1	R = 28.1-28.5
Tank 18 (B)	10.32 ± 0.59	8.53 ± 1.06	7.91 ± 0.12	32.1 ± 0	27.3 ± 0.14
	N = 12	N = 12	N = 12	N = 2	N = 2
	R = 9.5-11.0	R = 5.3-9.3	R = 7.6-8.1	R = 32.1-32.1	R = 27.2-27.4
Tank 20 (B)	10.36 ± 0.47	8.92 ± 0.29	7.95 ± 0.07	31.7 ± 0.42	27.8 ± 0.28
	N = 31	N = 31	N = 31	N = 2	N = 2
	R = 9.5-11.1	R = 8.3-9.6	R = 7.8-8.1	R = 31.4-32.0	R = 27.6-28.0

* N = number of readings; ** R = range of values.

APPENDIX 8

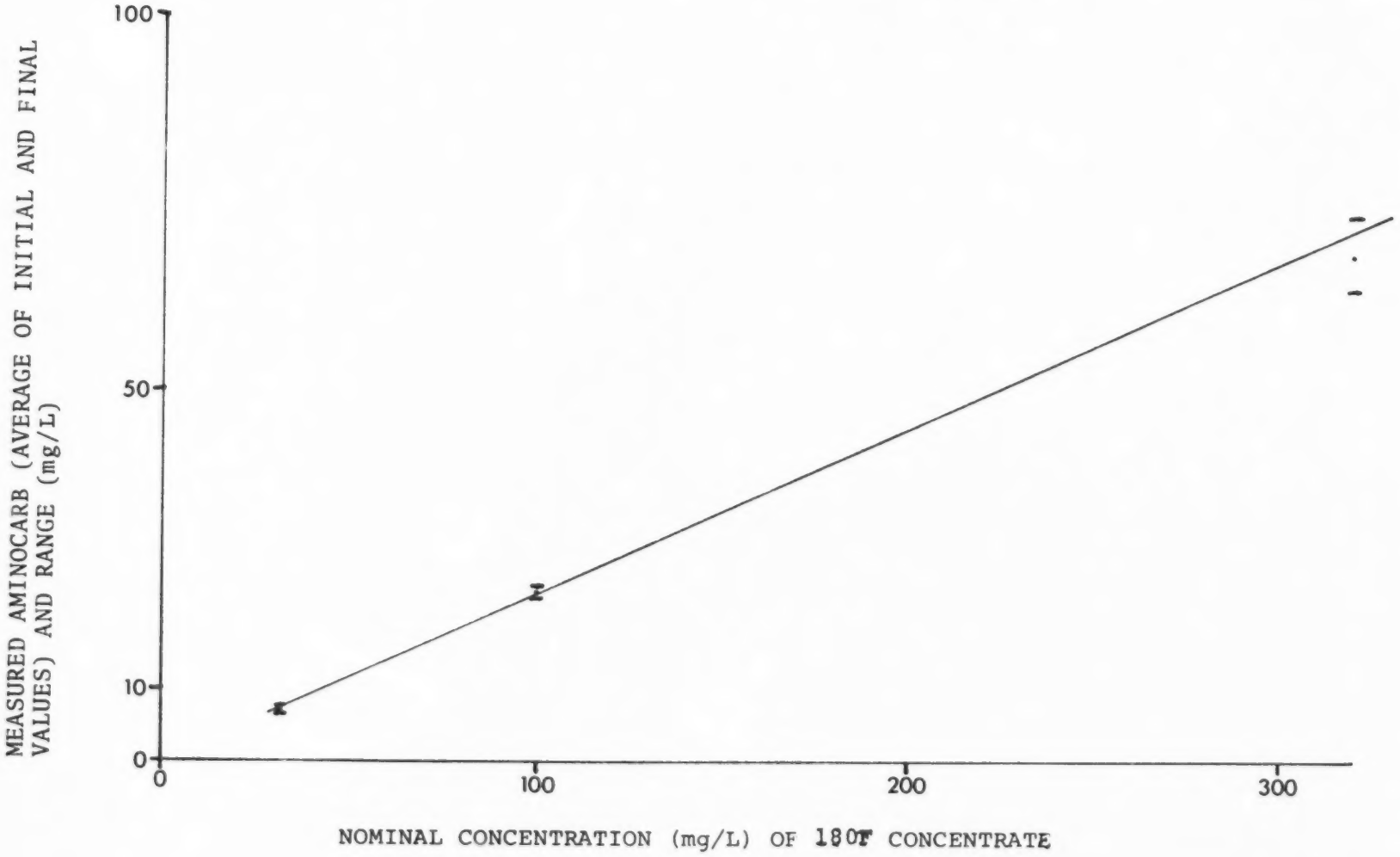
SUMMARY OF CHEMICAL MEASUREMENTS CONDUCTED ON TEST SOLUTIONS

Material Tested	Measured Concentrations of Aminocarb (mg/L)					Calculated Aminocarb Concentration (mg/L)
	Mean Initial Concentration $\bar{X} \pm$ S.D.	Mean Final Concentration $\bar{X} \pm$ S.D.	Combined Initial + Final Value			
			$\bar{X} \pm$ S.D.	N*	Range of Values	
Oil Formulation	70	66	68 ± 3.7	4	63-72	72.3
	20 ± 1.5	21 ± 1.0	21 ± 1.5	6	18-22	23.1
	7.0 ± 0.6	5.9 ± 1.6	6.4 ± 1.2	6	4.1-7.7	7.23
	1.8**	2.0**	1.9	2	1.8-2.0	2.31
Water Formulation	62 ± 7.0	60 ± 9.0	61 ± 7.3	6	51.0-69	60.4
	22 ± 1.5	21 ± 1.7	21 ± 1.4	6	20-23	19.3
	6.5	5.3 ± 2.1	5.8 ± 2.97	5	2.9-10	6.04
Matacil 180 F	74**	64**	69	2	64-74	77.8
	23**	24**	23.5	2	23-24	24.3
	7.2**	7.0**	7.1	2	7.0-7.2	7.78

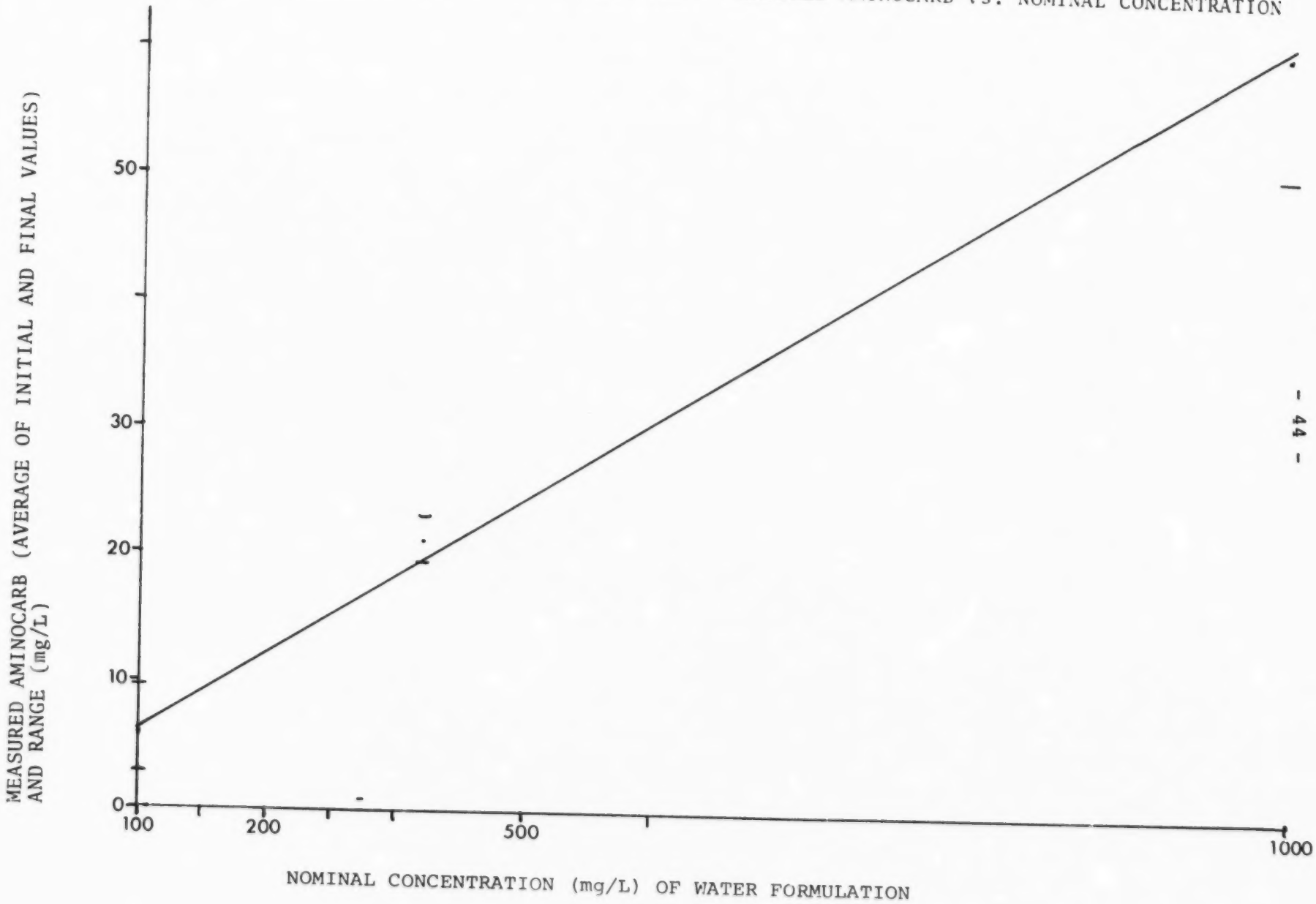
* N = Number of measurements.

** One value only.

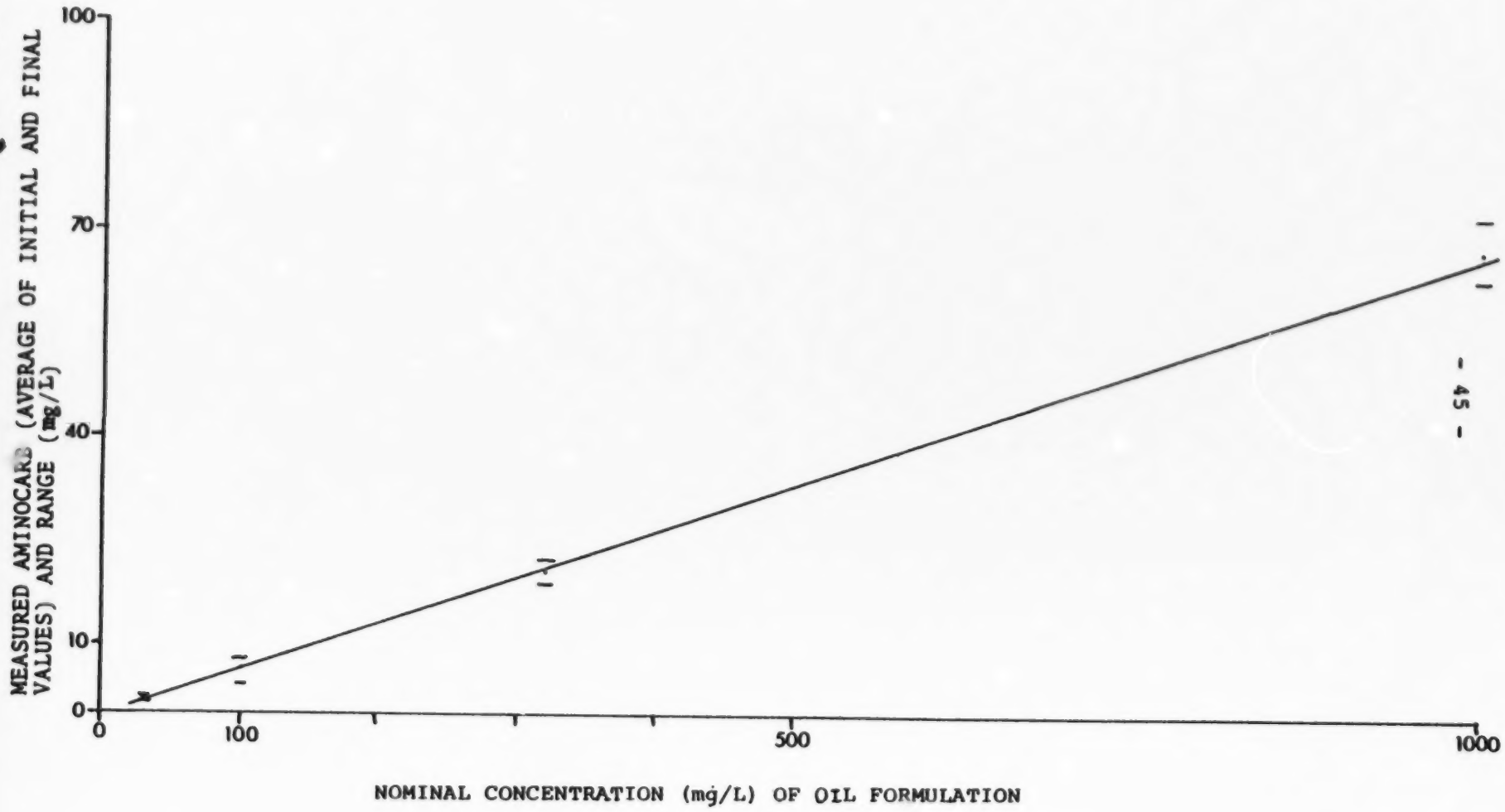
APPENDIX 9. MATACIL 180F CONCENTRATE: MEASURED AMINOCARB AGAINST NOMINAL CONCENTRATION



APPENDIX 10. MATACIL 180F WATER FORMULATION: MEASURED AMINOCARB vs. NOMINAL CONCENTRATION



APPENDIX 11. MATACIL 180F OIL FORMULATION: MEASURED AMINOCARB vs. NOMINAL CONCENTRATION



POOR

QUALITY

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